



Response of *Eretmocerus eremicus* to skylight and plant cues in a vertical flight chamber

J. L. Blackmer¹ & D. Cross²

¹Western Cotton Research Laboratory, USDA-ARS, 4135 E. Broadway Rd., Phoenix, AZ 85040, USA; ²Department of Entomology, The University of Arizona, Tucson, AZ 85721, USA

Accepted: May 22, 2001

Key words: flight behavior, visual cues, parasitoid, whiteflies, *Eretmocerus* sp. nr. *californicus*

Abstract

In the southwestern United States, *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) is a native parasitoid of the *Bemisia* complex (Homoptera: Aleyrodidae). However, little information currently exists on its potential as a biological control agent of whiteflies or on the factors that influence its tendencies to disperse. In this study, we examined the flight behavior of male and female *E. eremicus* in response to skylight (here simulated by a mercury-vapor lamp) and plant cues (a 550-nm filtered light) in a vertical flight chamber. Approximately 90% of the parasitoids took off in response to the skylight cue. Both sexes were capable of sustained flights in excess of 60 min; however, males had higher rates of climb than females (3.31 ± 0.17 and 2.63 ± 0.19 cm s⁻¹, respectively). When a plant cue was presented during the parasitoid's phototactic flight, four relatively distinct responses were observed. Fifty-one percent of the individuals responded to the plant cue throughout their flight by flying toward or by landing on the cue. The majority of these parasitoids were females. Approximately 12% of the wasps exhibited an intermittent, positive response to the plant cue. Twenty percent exhibited a 'migratory' response. These parasitoids, which were predominantly males, failed to respond to the plant cue until they had flown for a considerable period. Finally, 17% failed to respond to the target during their flight. Approximately 37% of the individuals that showed a positive response to the plant cue actually landed on it and the majority of these were female. The differential response to the plant cue by male and female parasitoids could be, in part, because females are driven to locate hosts in which to oviposit, and males are driven to find mates.

Introduction

Insect parasitoids use visual, acoustic, and olfactory cues to locate their prey (Vinson, 1976; Sugimoto et al., 1988; Hare & Luck, 1994; Heinz & Parrella, 1998). The olfactory cues used during host location and selection have been studied extensively. However, rather limited information exists on the other potential modalities that may be involved (Goff & Nault, 1984; Gregory, 1985; van Alphen & Vet, 1986). Prey location involves a sequence of steps, the first of which is host-habitat location. The plant often serves as one of the first cues for the parasitoid in the process that leads to host finding. In augmentative and inundative biological control programs, it is important to understand how the parasitoid locates its host and how it will

respond to its new environment. An understanding of the distribution of the host is also imperative. Whether the host tends to occur in aggregations or is evenly dispersed and how such distribution patterns influence the behavior of the parasitoid are critical.

Eretmocerus spp. have been used in several biological control programs (e.g., Onillon, 1990; Heinz & Parrella, 1998), and in the desert southwest *Eretmocerus eremicus* Rose and Zolnerowich is being evaluated as a biological control agent against *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) (Simmons & Minkenberg, 1994; Bellamy & Byrne, 2001). Newly released parasitoids will find themselves in an unfamiliar habitat, and due to the aggregated spatial distribution of whiteflies (Naranjo, 1996), a considerable distance may separate these wasps from the

nearest host. The female must locate a suitable host in a short period, or have the ability to emigrate to a new habitat. Searching efficiency, tenure time, and propensity to disperse are important considerations under these conditions.

In the present paper, we examined the flight potential of male and female *E. eremicus* when presented skylight and plant cues in the absence of whitefly or olfactory stimuli. Here we specifically focused on the transition from migratory behavior to behaviors associated with host-habitat location where the parasitoid could encounter its prey. Such behavioral transitions are comparable to what parasitoids experience as they move among crops in search of their hosts. The implications of our findings to biological control programs are discussed.

Materials and methods

Colony source and maintenance. The colony of *E. eremicus* used in our flight studies was established from collections of parasitized *B. tabaci* nymphs found on *Hibiscus* sp. growing in Phoenix, AZ. The identification of *E. eremicus* was provided by Mike Rose at Montana State University, and subsequently confirmed by polymerase chain reaction (PCR) technique. This species has been referred to as *E. haldemani*, *E. californicus* or *E. sp. nr. californicus* (Rose & Zolnerowich, 1997). *Eretmocerus eremicus* was reared on *B. tabaci* (Biotype B) on cotton, *Gossypium hirsutum* L. (c.v. 'Stoneville 853'), in a greenhouse located at The University of Arizona Agricultural Center, Tucson, AZ. To maintain a continuous supply of parasitoids, fresh cotton plants with unparasitized whitefly nymphs, were rotated into the greenhouse as needed. Colonies were maintained at $31 \pm 5^\circ\text{C}$ and between 48–88% r.h. with a L13:D11 photoperiod.

Response to skylight and plant cues. Flight behavior of *E. eremicus* was examined in a vertical flight chamber (Blackmer & Phelan, 1991; Blackmer & Byrne, 1993). A Philips 400 W mercury-vapor lamp was suspended above the chamber to simulate skylight. To examine the wasps' response to plant cues, the chamber was equipped with a 550 ± 2 nm narrow-band interference filter (50 mm diameter, 03 FIV 008, Melles Griot, Irvine, CA). The filter was positioned in the sidewall of the chamber, 15 cm from the ceiling, and was illuminated for 3 s during each minute of flight by a 50-W halogen bulb (General Electric) that

was connected to a 6-min repeating Tork timer (Model 8061, Mount Vernon, NY). The halogen bulb was mounted directly behind the filter on the outside of the flight chamber. The visual target (= filter) was opposite the flight zone (10–15 cm below the light window) of the insect during its phototactic flight. Wasps never responded to the target unless it was illuminated.

The chamber was equipped with a 100VT hot-wire airflow meter (Davis Instr., Baltimore, MD) connected to a strip-chart recorder (Soltec DB-1, San Fernando, CA), which measured air speed, providing a direct record of the insect's rate of climb and an indirect measure of its photokinetic response. Each presentation of the visual target was noted on the recording using a mark-trace key that produced a positive pulse. Additionally, the degree of displacement of the insect toward the target was measured by using a grid system (80×80 cm) that was superimposed onto the back wall of the flight chamber. If the wasp came within 15 cm of the plant cue, it was considered a positive response, as this required a directed movement that took the insect a minimum of 10 cm outside its normal flight pattern when flying toward the skylight cue.

All tests were conducted between 09:00 and 14:00 during the months of April and May with wasps that had been maintained in culture for less than one year. Parasitoids were flown only once, and had experienced host and plant cues before flight tests. According to Hunter et al. (1996), these wasps mate shortly after emergence, so we have assumed that most individuals were mated. Before each trial, wasps were collected from the colony and placed in gelatin capsules. The gender was determined and then the capsule was placed inside the flight chamber for a preconditioning period of approximately 30 min. At the onset of the test, the capsule was opened and the wasp was given 5 min to initiate flight. Wasps that failed to exhibit phototactic orientation to the overhead light or that did not take off were eliminated. For wasps that flew, the downward airflow into the flight chamber was controlled to maintain the insect in flight 10–15 cm below the light window. Sixty-three individuals (32 males and 31 females) were flown until they ended their flight by landing on the sidewall or floor of the chamber. The flight chamber was maintained at $27 \pm 1^\circ\text{C}$ and 28% r.h.

T-tests were used to compare flight duration and rates of climb for male and female parasitoids. Flight durations were transformed ($\log(y + 1)$) before analysis to meet the requirements of normality and homogeneity of variance. Male and female responses to the

plant cue were compared by χ^2 -test to determine if there were significant deviations from expected 50:50 distributions.

Results

Approximately 90% of the parasitoids took off in response to the skylight cue. Most flights were relatively stable in terms of rates of climb, as can be seen in the representative strip-chart recordings (Figures 1A–C) and from observations of the tight flight patterns in both the horizontal and vertical dimensions within the chamber (pers. observ.). The initial rates of climb for the first 3 minutes of flight varied from 1.5 to 10 cm s⁻¹ and were higher for males than for females (mean \pm SEM, 3.31 \pm 0.17 and 2.63 \pm 0.19 cm s⁻¹, respectively; $t = -2.51$, $P = 0.014$, $df = 64$). Both male and female parasitoids were capable of sustained flights in excess of 60 min with an average flight time of 15.6 \pm 3.8 min for males and 7.8 \pm 2.6 min for females. Even though male and female flight durations were not significantly different ($t = 1.72$, $P = 0.09$, $df = 61$), the fact that 32.2% of the males flew for longer than 20 min, while only 13.8% of the females did so, could be important biologically and ecologically.

When the plant cue was presented during the parasitoid's phototactic flight, four relatively distinct responses were observed (Figure 2). In the first category, individuals responded to the plant cue throughout their flights by either flying towards or by landing on the cue (Figures 1A and 2). Approximately 51% of the individuals exhibited this type of response, with the majority being female (71.4%, $\chi^2 = 3.86$, $df = 1$, $P < 0.05$). This type of response is typical of insects that are engaging in host-seeking behavior. A smaller percentage (12%) of individuals exhibited a mixed response (intermittently positive) to the plant cue (Figures 1B and 2), and the percentage of male and female parasitoids exhibiting this type of response was similar (40 and 60%, respectively). A third behavioral category, exhibited by approximately 20% of the parasitoids, would best be categorized as migratory (*sensu* Kennedy, 1961). These parasitoids failed to respond to the plant cue until they had flown for a considerable period (Figures 1C and 2). A significantly higher percentage of males exhibited this type of response (87.5 vs. 12.5%, $\chi^2 = 4.50$, $df = 1$, $P < 0.05$). Finally, about 17% of the parasitoids failed to respond to the target before they ended their flight and most

of these were male (71.4%). Approximately 37% of the individuals that showed a positive response to the plant cue, landed on it and most of these were females (73.7 vs. 26.3%, $\chi^2 = 4.26$, $df = 1$, $P < 0.05$). If the parasitoid landed on the cue, the tenure time was always less than 10 s.

Discussion

In the United States, *E. eremicus* and *Encarsia formosa* Gahan are commercially available for biological control of whiteflies. *Encarsia formosa* has been used extensively in greenhouse situations and consequently a great deal is known about its foraging behavior. Much less is known about *E. eremicus*. However, Hoddle et al. (1998) found that under greenhouse conditions *E. eremicus* was better at detecting whitefly-infested leaves and killed more nymphs than *E. formosa*, suggesting that under their conditions, *E. eremicus* might be a better choice as a biological control agent against whiteflies. In agricultural settings, parasitism rates by native aphelinid parasitoids (the majority of which are now believed to have been *E. eremicus*) have ranged as high as 60–90% in late summer (Gerling, 1966; Natwick & Zalom, 1984; Bellows & Arakawa, 1988). Simmons & Minkenberg (1994) demonstrated that augmentative releases of *E. eremicus* could sufficiently control whitefly populations. But these studies involved caged plants and consequently prevented both immigration and emigration of whiteflies and parasitoids.

As a component of the host-searching process, flight capacity and dispersal tendencies are often overlooked, perhaps because of the difficulties in studying the movement of such small insects. Nevertheless, being able to predict when a parasitoid might leave a patch would be an extremely important consideration when evaluating the efficacy of the biological control agent. Farias & Hopper (1997) reported that the aphelinid parasitoid, *Aphelinus asychis* Walker, was a weak flier and was unable to orient upwind. However, we found that both male and female *E. eremicus* were able to sustain directed flight toward a skylight cue against a 10 cm s⁻¹ downward draft of air. Flights were very stable, and for many individuals, flight durations exceeded 20 min. Bellamy & Byrne (2001) examined the factors that influence dispersal of *E. eremicus* in the field and laboratory and although rearing conditions and behavioral history of the parasitoids were quite different from ours, there are some important simi-

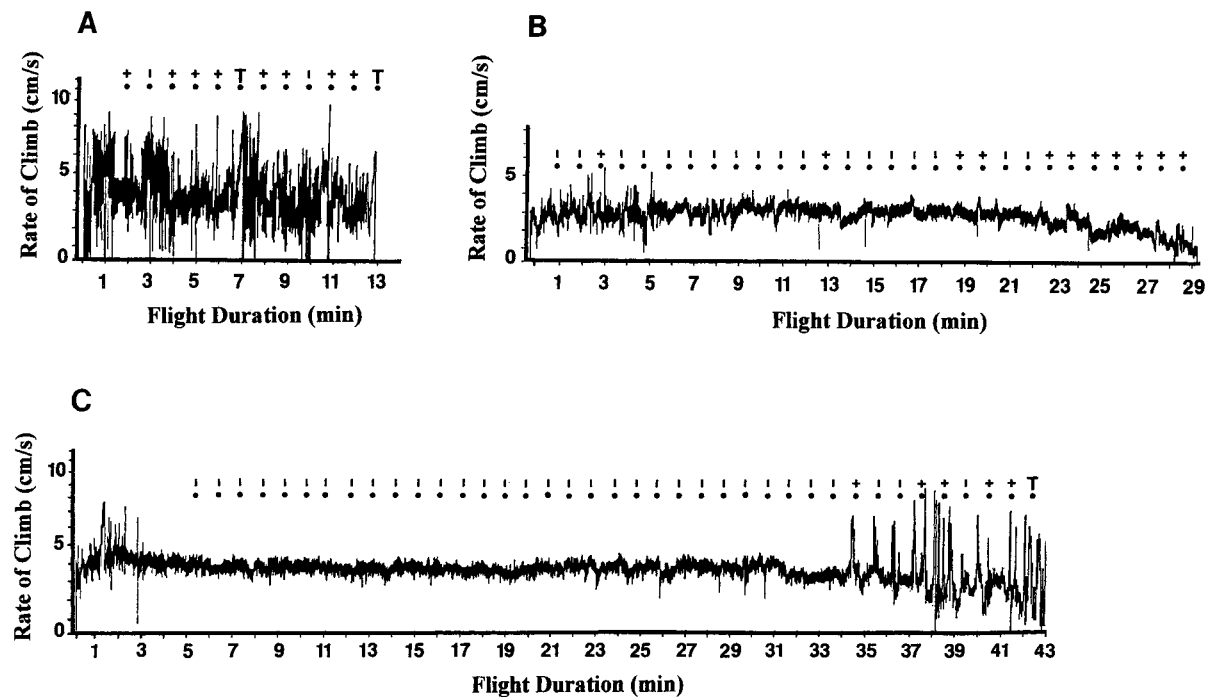


Figure 1. Representative strip-chart recordings of *Eretmocerus eremicus* flights in a vertical flight chamber: A) female parasitoid exhibiting a trivial or foraging-type response, B) male parasitoid exhibiting an intermittent, positive attraction, and C) a male exhibiting a 'migratory' response to a plant cue; • = plant cue illuminated, + = parasitoid flew toward the plant cue, - = parasitoid did not approach the plant cue, T = parasitoid landed on the plant cue.

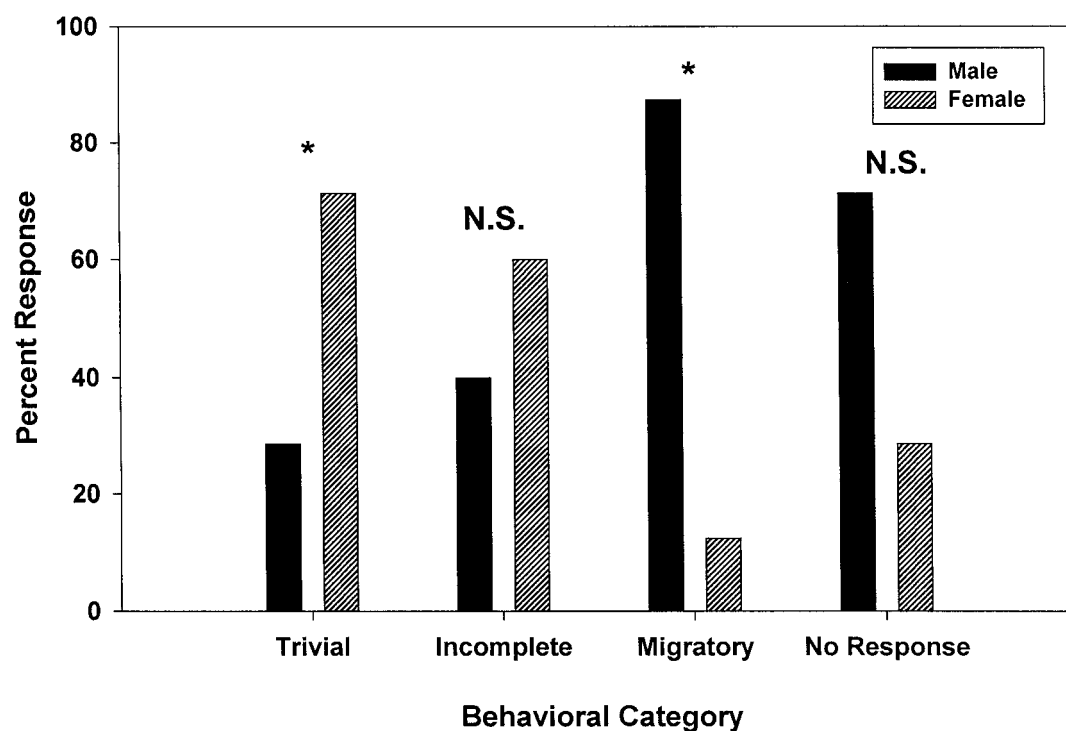


Figure 2. Percentage of male and female *Eretmocerus eremicus* that responded to the plant cue throughout their flight (= trivial or foraging-type response), that showed an intermittent, positive response to the plant cue, that only responded to the plant cue at the end of their flight (= migratory flight) or that exhibited no response to the plant cue. * $P < 0.05$.

larities. Their findings also suggest that *E. eremicus* are capable of directed flights, with gender and mating status being important determining factors in flight duration.

When *E. eremicus* was presented conflicting cues (those that evoke migratory versus those that evoke settling behaviors) during their flight in a vertical flight chamber, 63% flew toward the plant cue (550-nm interference filter) immediately or shortly after it was presented. These individuals were almost exclusively female and exhibited a response that in nature would be associated with foraging. Wäckers (1994) stated that parasitoids foraging for hosts demonstrate innate responses to unreliable indirect stimuli such as the plant image and color, which by themselves are poor predictors of host presence. However, when you consider that the highly reliable, direct host stimuli are often undetectable at longer ranges, the need for responses to less reliable (but more detectable) stimuli may become genetically programmed.

Several studies have demonstrated that parasitoids are attracted to cues that supposedly simulate the plant, such as yellow sticky traps or transmitted light with a spectral range of 515–550 nm (Vater, 1971; Goff & Nault, 1984; Hoelmer et al., 1998). This attraction, however, apparently varies depending on species, gender, time of sampling and host availability (Webb & Smith, 1980; van de Veire & Vacante, 1984; Hoelmer et al., 1998). Wäckers (1994) found that food-deprived *Cotesia rubecula* Marshall (Hymenoptera: Braconidae) landed more often and spent more time searching on yellow targets, while sugar-fed individuals displayed a higher overall foraging activity, without reacting preferentially to yellow. We found that there is a gender-related difference in response to plant cues for *E. eremicus*, with females being much more responsive in terms of displacement towards and landings on the plant cue. Males seldom landed on the plant cue but were more likely to respond to it after an extended 'migratory' flight. Differences between the sexes in tendencies to disperse or engage in host-seeking behaviors could be due to physiological constraints (i.e., egg load, flight fuels, mating status, etc.) or to differences in resources that are utilized (i.e., a host for oviposition, nectar source, or a mate). In all cases where the parasitoid landed on the visual target, tenure time was less than 10 s. Tenure time in other parasitoid spp. is influenced by the presence of whitefly nymphs and honeydew (Shimron et al., 1992; van Roermund & van Lenteren, 1995). Apparently, *E. eremicus* was able to quickly determine that

whiteflies were absent from the plant cue. In field trials, Bellamy & Byrne (2001) also found that female *E. eremicus* readily left field plots when whiteflies were absent or in low numbers. Additional field and laboratory studies are needed so that we might better understand the underlying mechanisms behind these gender-based differences in responses to plant cues and in flight duration.

Acknowledgements

We would like to thank David Byrne for providing the opportunity and space to conduct these studies and David Byrne, Steve Naranjo, Cesar Rodriguez-Saona, and Dan Gerling for critical reviews of previous versions of this manuscript, Mike Rose for identification of *E. eremicus* and Katie Zigweid for subsequent PCR analysis for verification of the species.

References

- Alphen, J. J. M. van & L. E. M. Vet, 1986. An evolutionary approach to host finding and selection. In: J. K. Waage & D. J. Greathead (eds), *Insect Parasitoids*. Academic Press, London, pp. 23–61.
- Bellamy, D. & D. N. Byrne, 2001. Effects of gender and mating status on self-directed dispersal by the whitefly parasitoid *Eretmocerus eremicus*. *Ecological Entomology*, in press.
- Bellows, T. S. & K. Arakawa, 1988. Dynamics of preimaginal populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Eretmocerus* sp. (Hymenoptera: Aphelinidae) in southern California cotton. *Environmental Entomology* 17: 483–487.
- Blackmer, J. L. & P. L. Phelan, 1991. Behavior of *Carpophilus hemipterus* in a vertical flight chamber: transition from phototactic to vegetative orientation. *Entomologia Experimentalis et Applicata* 58: 137–148.
- Blackmer, J. L. & D. N. Byrne, 1993. Flight behaviour of *Bemisia tabaci* in a vertical flight chamber: effect of time of day, sex, age and host quality. *Physiological Entomology* 18: 223–232.
- Farias, A. M. I. & K. R. Hopper, 1997. Responses of female *Aphelinus asychis* (Hymenoptera: Aphelinidae) and *Aphidius matricariae* (Hymenoptera: Aphidiidae) to host and plant-host odors. *Environmental Entomology* 26: 989–994.
- Gerling, D., 1966. Studies with whitefly parasites of southern California II. *Eretmocerus californicus* Howard (Hymenoptera: Aphelinidae). *Canadian Entomologist* 98: 1316–1329.
- Goff, A. M. & L. R. Nault, 1984. Response of the pea aphid parasite *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) to transmitted light. *Environmental Entomology* 13: 595–598.
- Gregory, W. A., 1985. In-flight response of citrus-inhabiting Aphelinidae (Hymenoptera) to trap colors and plant structures. Ph.D. Dissertation, University of California, Riverside.
- Hare, J. D. & R. F. Luck, 1994. Environmental variation in physical and chemical cues used by the parasitoid wasp, *Aphytis melinus*, for host recognition. *Entomologia Experimentalis et Applicata* 72: 97–108.
- Heinz, K. M. & M. P. Parrella, 1998. Host location and utilization by selected parasitoids of *Bemisia argentifolii* (Homoptera:

- Aleyrodidae): Implications for augmentative biological control. *Environmental Entomology* 27: 773–784.
- Hoddle, M. S., R. G. Van Driesche, J. S. Elkinton & J. P. Sanderson, 1998. Discovery and utilization of *Bemisia argentifolii* patches by *Eretmocerus eremicus* and *Encarsia formosa* (Beltsville strain) in greenhouses. *Entomologia Experimentalis et Applicata* 87: 15–28.
- Hoelmer, K. A., W. J. Roltsch, C. C. Chu & T. J. Henneberry, 1998. Selectivity of whitefly traps in cotton for *Eretmocerus eremicus* (Hymenoptera: Aphelinidae), a native parasitoid of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Environmental Entomology* 27: 1039–1044.
- Hunter, M. S., M. F. Antolin & N. Rose, 1996. Courtship behavior, reproductive relationships, and allozyme patterns of three North American populations of *Eretmocerus* nr. *californicus* (Hymenoptera: Aphelinidae) parasitizing the whitefly *Bemisia* sp., *tabaci* complex (Homoptera: Aleyrodidae). *Proceedings of the Entomological Society of Washington* 98: 126–127.
- Kennedy, J. S., 1961. A turning point in the study of insect migration. *Nature* 186: 348–350.
- Naranjo, S. E., 1996. Sampling *Bemisia* for research and pest management applications. In: D. Gerling & R. T. Mayer (eds), *Bemisia* 1995: Taxonomy, Biology, Damage, Control and Management. Intercept, Andover, UK, pp. 209–224.
- Natwick, E. T. & F. G. Zalom, 1984. Surveying sweetpotato whitefly in the Imperial Valley. *California Agriculture* 38(3/4): 11.
- Onillon, J. C., 1990. The use of natural enemies for the biological control of whiteflies. In: D. Gerling (ed.), *Whiteflies: their Bio-nomics, Pest Status and Management*. Intercept, Andover, UK, pp. 287–314.
- Roermund, H. J. W. van & J. C. van Lenteren, 1995. Foraging behaviour of the whitefly parasitoid *Encarsia formosa* on tomato leaflets. *Entomologia Experimentalis et Applicata* 76: 313–324.
- Rose, M. & G. Zolnerowich, 1997. *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) in the U.S., with descriptions of new species attacking *Bemisia (tabaci)* complex (Homoptera: Aleyrodidae). *Proceedings of the Entomological Society of Washington* 99: 1–27.
- Shimron, O., A. Hefetz & D. Gerling, 1992. Arrestment responses of *Eretmocerus* species and *Encarsia deserti* (Hymenoptera: Aphelinidae) to *Bemisia tabaci* honeydew. *Journal of Insect Behavior* 5: 517–526.
- Simmons, G. S. & O. P. J. M. Minkenberg, 1994. Field cage evaluation of augmentative biological control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) in southern California cotton with the parasitoid *Eretmocerus* nr. *californicus* (Hymenoptera: Aphelinidae). *Environmental Entomology* 23: 1552–1557.
- Sugimoto, T., Y. Shimono, Y. Hata, A. Nakai & M. Yahara, 1988. Foraging for patchily-distributed leaf-miners by the parasitoid, *Dapsilarthra rufiventris* (Hymenoptera: Braconidae) III. Visual and acoustic cues to a close range patch location. *Applied Entomology and Zoology* 23: 113–121.
- Vater, G., 1971. Über Ausbreitung und Orientierung von *Di-aeretiella rapae* (Hymenoptera, Aphidiidae) unter Berücksichtigung der Hyperparasiten von *Brevicoryne brassicae* (Homoptera, Aphididae). *Zeitschrift für Angewandte Entomologie* 68: 187–225.
- Veire, M. van de & V. Vacante, 1984. Greenhouse whitefly control through the combined use of the colour attraction system with the parasite wasp *Encarsia formosa* (Hym.: Aphelinidae). *Entomophaga* 29: 303–310.
- Vinson, S. B., 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21: 109–133.
- Wäckers, F. L., 1994. The effect of food deprivation on the innate visual and olfactory preferences in the parasitoid *Cotesia rubecula*. *Journal of Insect Physiology* 40: 641–649.
- Webb, R. E. & F. F. Smith, 1980. Greenhouse whitefly control of an integrated regimen based on adult trapping and nymphal parasitism. *IOBC/WPRS Bulletin* III/3: 235–246.